PY-GC-ION TRAP DETECTION OF SORGHUM GRAIN POLYPHENOLS (*syn.* VEGETABLE TANNINS): PRELIMINARY RESULTS

Guido C. Galletti
Centro di Studio per la Conservazione dei Foraggi - C.N.R.
via Filippo Re, 8
40126 Bologna, Italy

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ABSTRACT

Polyphenols (tannins) from sorghum grains with a high tannin content were analyzed by pyrolysis-gas chromatography-ion trap detector mass spectrometry (PY-GC-MS). Pyrolysis at 600 °C produced high relative percentages (around 50%) of catechol (1,2-dihydroxybenzene), and minor amounts of other phenolic compounds. Catechol was also the main fragment in the pyrogram of catechin (a monomeric unit of tannins). The absence of significant levels of catechol in the pyrogram of non-tannin polyphenols (such as lignin), and its relative lower abundance in that of low-tannin containing sorghum grains suggests the use of catechol as a remarkable characteristic fragment for quali- quantitative analysis of tannins by PY-GC-MS.

INTRODUCTION

Polyphenols (*syn.* vegetable tannins) can be divided into two classes, namely hydrolizable and condensed or non-hydrolizable tannins. The hydrolizable tannins are esters of mainly glucose with hydroxyphenolic acids such as gallic acid. The condensed tannins are polymers of flavanoid precursors such as catechin (scheme 1) (1).

Vegetable tannins influence the characteristics of many plant products - their taste, palatability, nutritional value, pharmacological and toxic effects, and their microbial decomposition - because of their ability to complex strongly with proteins, carbohydrates, nucleic acids, alkaloids and minerals (2,3,4).

Structure elucidation of monomeric and oligomeric (up to 5-6 units) procyanidins has been accomplished by ^{1}H- and ^{13}C-NMR (5), and Fast Atom Bombardment Mass Spectrometry (6). Classical methods for routine quantitative analysis have included colorimetric (7,8) and protein precipitation assays (9), but their validity has been questioned (10).

Tannins have been chromatographed by HPLC (11) and gel permeation (12). Gas chromatography (GC) is unfeasible due to their large molecular weight, polarity and thermal lability. However, molecules with these chemical features are in principle suitable to be degraded by pyrolysis (PY), an effective technique for the study of complex, non-volatile samples, which can be integrated with a gas chromatograph-mass spectrometer (PY-GC-MS) to provide a rapid analysis of the degradation products, and hence the characterization of the original sample.

This paper reports on the GC-MS analysis of the pyrolysis products of tannins isolated by gel permeation from sorghum grain, and of catechin as a reference compound. It is anticipated that these preliminary results indicate whether pyrolysis can provide characteristic fragments of diagnostic significance for the recognition of the non-volatile tannins by gas chromatography without the need for off-line degradation and derivatization or mass spectrometry.

EXPERIMENTAL

Extraction and gel permeation.

A detailed procedure for tannin extraction and purification has been described elsewhere (12). Briefly, a 100-g sample of ground sorghum grain was defatted in Sohxlet with diethyl ether. The residue was transferred into a dark bottle and extracted overnight at +1 °C under rotary shaking with acetone (3x300 ml). The residue was extracted under the same conditions with methanol (3x300 ml). The methanolic extracts were combined and the solvent was distilled off under vacuum in a rotary evaporator (T: 35 °C). A 500-mg aliquot of this crude extract was suspended in methanol (7 mL) and the slurry was applied onto a column packed with Sephadex LH 20 (35x3 cm). The column was eluted at 2 mL/min (detection 340 nm) with 95% ethanol (800 mL) to remove some coloured non-phenolic substances, then with acetone/water (7/3, 640 mL) to collect the brown polyphenolic fraction retained at the top of the column. The acetone/water solvent was evaporated in rotary evaporator under reduced pressure (T: 40 °C). The tannin was dried over P_2O_5 and stored at - 25 °C until analysis.

Pyrolysis-gas chromatography-mass spectrometry (PY-GC-MS).

About 1 mg of tannin was pyrolyzed using a CDS Pyroprobe 100 equipped with a platinum coil probe and a quartz sample holder. Catechin was pyrolyzed by applying 10 µL of a methanolic solution (10 mg/mL) in the quartz tube. The pyrolyzer was interfaced with a GC-MS system consisting of a Varian 3400 gas chromatograph coupled to a Finnigan MAT Ion Trap Detector (ITD) model 800 mass spectrometer and operating under the following conditions: injector (split 1/120) at 220 °C; SPB-5 column (30 m x 0.32 mm i.d.) programmed from 50 to 300 °C at 10 °C/min (He: 1 mL/min); transfer line at 220 °C; mass spectra recorded at 70 eV under the usual ITD software release 4.0 conditions.

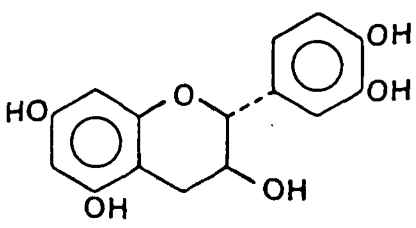
RESULTS AND DISCUSSION

Two sorghum grain commercial cultivars, i.e. Arval and Argence representative of high- and low tannin content, respectively, were used for the present work. According to the vanillin test (8), Arval tannin content was 5.30 (expressed as catechin equivalent percentage of original sample dry matter) and that of Argence was 0.10. Table 1 collects these figures, and the yields of crude extract and tannin after gel permeation expressed as percentage of original sample dry matter and crude extract, respectively.

The total ion chromatograms (TIC) of the Arval and Argence tannin fractions after pyrolysis at 600 °C are shown in figures 1 and 2, respectively. Table 2 collects the eight most abundant ions in the mass spectra of the main peaks. The identification proposed for most of the pyrolysis fragments is reported in table 3. The fragmentation under pyrolysis was similar for the two samples, but the quantitative analysis (relative percentages) was substantially different (table 3). Catechol (identified by both mass spectrometry and injection of pure standard) was the main fragment (55.4%) in the pyrogram of Arval tannin (fig. 1, peak no. 24), whereas it was only 4.58% in that of

Table 3. Relative percentage and proposed identification of the main pyrolysis peaks in the two sorghum tannins Arval and Argence.

#	Arval	Argence	Compound
1	9.00	14.15	carbon dioxide
2	trace	trace	unknown
3	0.82	-	acetone
4	5.28	6.88	unknown
5	6.47	17.40	propylacetate
6	5.95	-	3-methylfuran
7	0.56	4.75	2-furanol, tetrahydro-2,3-dimethyl
8	0.34	0.94	unknown
9	0.67	0.27	3-furaldehyde
10	0.94	1.42	pyridine
11	0.20	0.15	toluene
12	1.28	0.71	unknown
13	1.16	-	unknown
14	0.33	-	unknown
15	0.30	0.39	unknown
16	1.51	17.69	phenol
17	1.09	0.88	unknown
18	0.22	1.17	unknown
19	0.51	1.80	4-methylphenol
20	0.62	3.81	guaiacol
21	0.45	0.28	unknown
22	0.17	0.36	unknown
23	0.52	2.53	ethylphenol
24	55.4	4.58	catechol
25	0.10	7.06	2-ethenylphenol
26	7.25	1.89	dihydroxytoluene



Scheme 1. Catechin (flavan-3-ol).

Table 1. Polyphenol estimation using the vanillin test (% of catechin equivalents (CE)), crude extract yield (% of original sample dry matter), and polyphenol yield (% of crude extract).

	CE	crude extract	polyphenol
Arval	5.30	2.40	43.0
Argence	0.10	1.81	3.4

Table 2. Eight-peak mass spectra of the pyrolysis fragments of Arval sorghum tannin.

#	scan	MW	m/z (%)							
1	142	44	44(100)	45(40)	43 (1)	-	-	-	-	-
2	147	-	43(100)	41(68)	42(38)	49(38)	45(36)	47(33)	48(30)	55(17)
3	151	58	58(100)	59(45)	42(44)	60(13)	44(12)	57(9)	40(7)	45(5)
4	155	-	43(100)	59(36)	42(13)	58(10)	41(4)	40(3)	44(3)	60(2)
5	169	102(0.2)	43(100)	61(25)	45(19)	42(15)	44(4)	41(1)	71(1)	59(1)
6	177	82	82(100)	53(91)	81(78)	50(43)	51(41)	52(18)	83(14)	54(12)
7	192	116	43(100)	75(12)	42(11)	57(7)	41(6)	44(6)	45(3)	40(2)
8	201	-	43(100)	45(58)	57(45)	74(34)	42(29)	73(27)	75(23)	41(22)
9	211	96	96(100)	95(94)	53(75)	51(56)	50(48)	43(39)	67(25)	52(22)
10	231	79	52(100)	79(91)	50(66)	43(62)	51(55)	80(37)	78(12)	53(11)
11	245	92	91(100)	92(50)	63(19)	65(18)	43(15)	41(11)	45(10)	50(10)
12	251	-	43(100)	85(25)	101(18)	100(14)	42(10)	45(4)	72(4)	44(3)
13	261	-	55(100)	43(60)	41(55)	83(48)	53(18)	42(17)	99(17)	98(13)
14	269	-	41(100)	55(76)	70(47)	43(36)	42(29)	69(25)	53(18)	56(12)
15	406	-	41(100)	53(53)	96(47)	42(44)	67(44)	55(38)	69(34)	81(34)
16	417	94	94(100)	66(65)	65(38)	63(23)	50(21)	40(17)	51(14)	53(12)
17	461	-	57(100)	41(99)	55(40)	43(39)	56(29)	70(27)	105(21)	83(20)
18	492	-	91(100)	45(83)	77(78)	128(72)	107(61)	108(61)	79(56)	69(50)
19	510	108	107(100)	108(85)	77(58)	79(50)	51(38)	50(31)	53(27)	63(23)
20	527	124	109(100)	81(99)	124(72)	53(54)	51(34)	52(33)	50(29)	63(14)
21	559	138	81(100)	43(40)	41(39)	54(30)	64(28)	42(25)	53(20)	138(10)
22	585	-	43(100)	55(30)	42(27)	107(15)	44(14)	51(13)	101(12)	69(12)
23	600	122	107(100)	77(43)	122(34)	41(22)	51(22)	53(17)	79(15)	50(14)
24	631	110	110(100)	63(38)	64(35)	53(30)	81(25)	51(18)	50(17)	82(17)
25	647	120	120(100)	91(97)	69(78)	41(48)	42(42)	57(23)	119(23)	73(22)
26	718	124	124(100)	78(75)	123(50)	51(42)	77(33)	67(20)	53(20)	106(18)

Argence tannin (fig. 2). In the latter, the main fragment was phenol (peak no. 17, 17.7%), which accounted for only 1.51% of Arval tannin TIC. Total phenolic content was also different in the pyrograms of the two tannins, i.e. 65.91% in Arval and 40.23% in Argence. The relative quantities of catechol were consistent with the results of the tannin colorimetric assay of the two sorghum cultivars reported in table 1, i.e. high percentage of catechol in Arval and low in Argence. In another work (13), it was also observed that catechol was present in non-significant amounts in the pyrogram of a non-tannin polyphenol such as lignin. The pyrolysis of catechin under the same conditions of tannins yielded catechol as the single degradation product (fig. 3). A black residue was observed in the quartz sample holder after pyrolysis, probably due to some polymerization reactions undergone by the other moiety of the molecule, which might explain the lack of other pyrolysis fragments. Increasing the temperature of pyrolysis up to 900 °C did not change the fragmentation. The reproducibility of triplicate pyrolysis of catechin was good (fig. 4).

CONCLUSION

PY-GC-MS analysis of condensed tannins containing catechin-like monomeric units shows catechol as main fragment. This compound is of diagnostic significance for condensed tannins not only because it is originated by a simple cleavage of one of its monomeric units, but also because it is peculiar of this class of polyphenols and not, for instance, of another class of polyphenols widespread in nature such as lignin, and because its formation under pyrolysis is quantitatively reproducible. Further studies are underway to screen a larger set of samples, to establish a quantitative correlation between the amount of catechin subjected to pyrolysis and the quantity of produced catechol, and to determine its range of linear response. If the preliminary results of the present work will be confirmed on a more quantitative basis, pyrolysis affords the prospect of a rapid and specific analysis of tannins by gas chromatography, a technique so far neglected for tannins, without the need of cumbersome sample preparation and derivatization.

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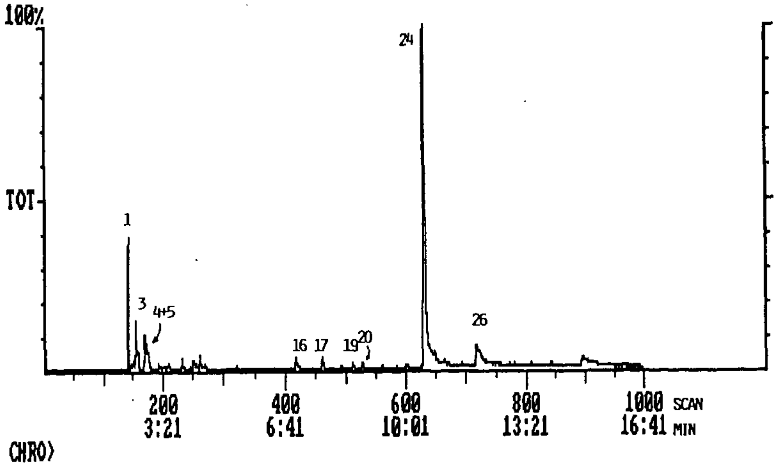

Fig. 1. Total ion chromatogram of Arval sorghum tannin after pyrolysis at 600 °C.

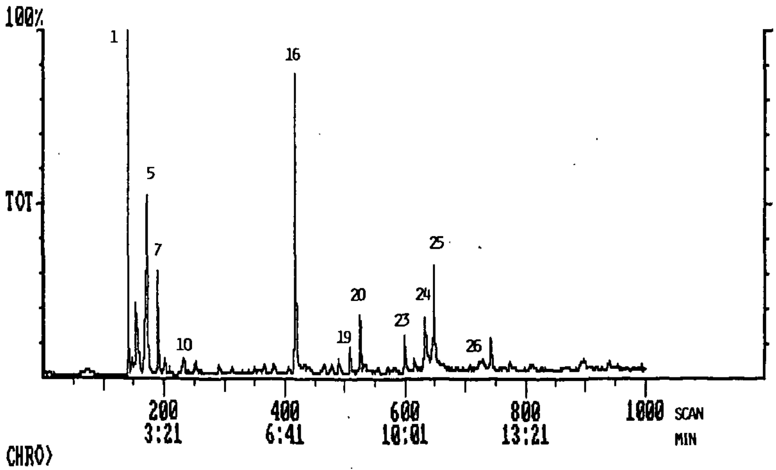

Fig. 2. Total ion chromatogram of Argence sorghum tannin after pyrolysis at 600 °C.

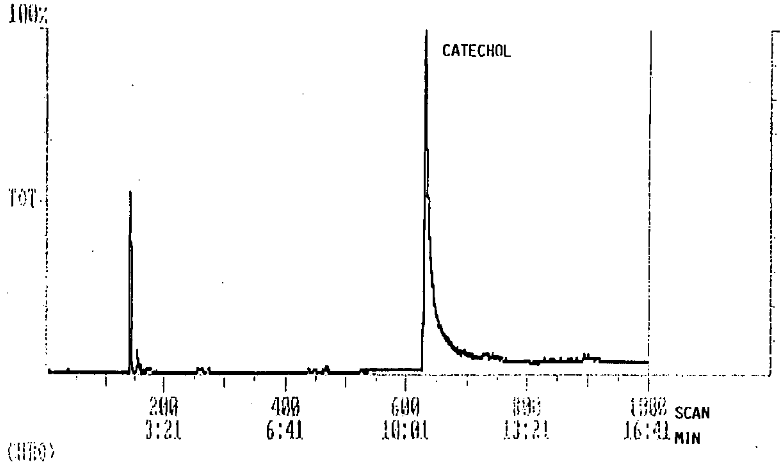

Fig. 3. Total ion chromatogram of catechin after pyrolysis at 600 °C.

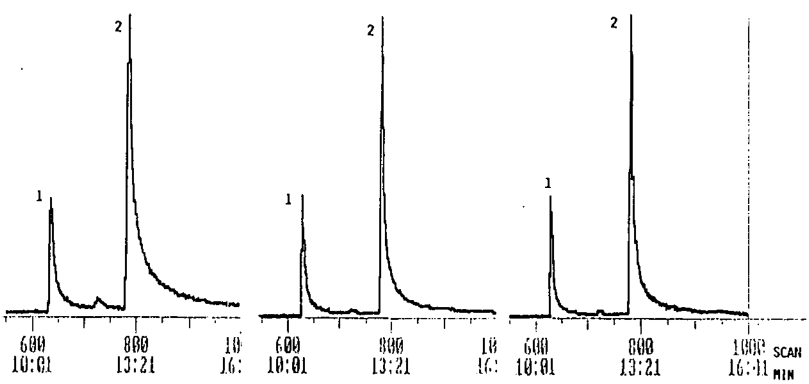

Fig. 4. Replicate pyrolysis of catechin under the same conditions of figure 1-3. (1) catechol, (2) p-hydroxybenzaldehyde which was added in view of its possible use as an internal standard in future quantitative experiments.